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Original Research Article

Bio-equivalence study of two tilmicosin phosphate formulations (Micotil 300® and Cozina 300®) in broiler chickens

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ABSTRACT

Background: The present study was designed to assess the comparative bio-equivalence of Micotil 300® and Cozina 300® in healthy broiler chickens after oral administration of both products in a dose of 15 mg tilmicosin base/kg body wt.
Methods: Twenty four broiler chickens were divided equally into two groups (12 chickens for each group). The first group was designed to study the pharmacokinetics of Micotil 300®, while the 2nd group was designed to study the pharmacokinetics of Cozina 300®. Each broiler chicken in both groups was orally administered with 15 mg tilmicosin/kg body wt. Blood samples were obtained from the wing vein and collected immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single oral administration.
Results: The disposition kinetics of Micotil 300® and Cozina 300® following oral administration of 15 mg tilmicosin/kg body wt revealed that the maximum blood concentration [C_{max}] were 1.73 and 1.67 µg/ml and attained at [t_{max}] of 2.01 and 2.04 hours, respectively.
Conclusions: Cozina 300® is bioequivalent to Micotil 300® since the ratios of C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ (T/R) were 0.96, 0.93 and 0.91 respectively. These are within the bio-equivalence acceptance range. Micotil 300® and Cozina 300® are therefore bioequivalent and interchangeable.

Keywords: Pharmacokinetics, Chickens, Tilmicosin

INTRODUCTION

Tilmicosin is a broad-spectrum bacteriostatic macrolide antibiotic synthesized from tylosin for veterinary use only. It has an antibacterial spectrum that is predominantly effective against *Mycoplasma* sp., *Pasteurella* sp. and various Gram-positive organisms.[1] It has been used extensively to treat respiratory disease in swine, cattle and sheep.[2-4] Tilmicosin is licensed for the treatment and control of respiratory diseases associated with *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale* and *Pasteurella multocida* in broiler chickens.[5-7] Tilmicosin exhibits good tissue penetration, reaching much higher concentrations in the lung than in the serum.[8]

The bio-equivalence studies play an important role in determining therapeutic efficacy to register the generic drug products according to the Food and Drug Administration (FDA) regulations.[9] Bio-equivalence is defined as statistically equivalent bioavailability between two products at the same molar dose of the therapeutic moiety under similar experimental conditions.[9,10] The drug products are said to be bioequivalent if they are pharmaceutical equivalents or pharmaceutical alternatives and if their rate and extent of absorption do not show a

significant differences statistically according to the FDA regulations.[9]

The aim of this study is to evaluate bio-equivalence of two solutions of tilmicosin (Micotil 300® and Cozina 300®) after oral administration of a single dose of 15 mg tilmicosin/kg body wt. in broiler chickens.

METHODS

Drugs

Micotil 300® is manufactured by Elanco-Animal Health, Gmbh, Germany). It is dispended as injectable solution. Each 1ml contains 300 mg tilmicosin (as phosphate) and it was used as reference product.

Cozina 300® is manufactured by Boston Company, Pharma Cure Division, Egypt, as injectable solution. Each 1 ml contains 300 mg tilmicosin (as phosphate) and it was used as test product.

Broiler chickens and experimental design

Twenty four healthy one day old broiler chickens were obtained from Benha private poultry farm, Egypt. They were kept individually in cages, within a ventilated, heated room (20˚C), and 23 hours of day light. They received a standard commercial ration free from any antibiotics for 30 days before starting the experiment to insure complete clearance of any anti-bacterial substances from their bodies. Water was offered *ad-libitum*.

Bio-equivalence study

Broiler chickens (30 days old and weighing 1.8-1.95 kg) were used to study the bio-equivalence of Micotil 300® and Cozina 300® after oral administration. Broiler chickens were divided into two groups. The 1st group (12 broiler chickens) was used to study the pharmacokinetics of Micotil 300®. The 2nd group (12 broiler chickens) was used to study the pharmacokinetics of Cozina 300®. Broiler chickens in the 1st group were administered orally (intra-crop) with Micotil 300® at a dose of 15 mg tilmicosin/kg body wt, while broiler chickens in the 2nd group were administered orally with cozina 300® at a dose of 15 mg tilmicosin/kg body wt.

Blood samples

Blood samples were obtained from the wing vein (1 ml) and collected in test tubes immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single oral administration (groups 1 and 2). Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of tilmicosin concentration. The serum samples were stored at −20˚C until analysis, and the assay was performed within a week of obtainment.

Analytical procedure

Rapid agar-diffusion assay for the quantitative determination of tilmicosin in small volumes of blood by using *Bacillus subtilis* (ATCC 6633) as a test organism.[11] Fresh stock solutions of tilmicosin at 250 µg/ml were made up in 0.1 M buffer (pH 6.0) for each set of assays. About 1 ml of the suspension of *Bacillus subtilis* (was added to 100 ml agar at 55 to 60ºC. The mixture was shaken thoroughly till complete mixing of the test organism with agar. Petri dishes (20×20 cm) were used; about 25 ml of inoculated medium were poured to each dish by using sterile cylinder. After complete solidification, six wells were made on the surface of inoculated agar using stainless steel cylinder. The wells of each plate were filled with the serum sample. The plates were incubated at 37˚C for 16 to 18 hours. The diameter of each inhibition zone was measured.

The calibration curves of serum were prepared with different concentrations between 0.1 and 100 µg/ml using blank chickens serum. Thereafter, the diameters of inhibition zones were measured with the aid of a transparent rule to the nearest millimeter. Each sample was replicated three times and analyzed similarly. The plot of tilmicosin serum concentrations versus diameters of inhibition zone was linear with a correlation coefficient of 0.971. Serum concentrations of tilmicosin were determined by comparing the zone of inhibition diameters with the standard curve. The absence of interfering endogenous compounds was demonstrated in antibacterial-free plasma obtained at time 0 (pretreatment) which showed no visible zone of inhibition around the impregnated disks. The limit of quantification (LOQ) defined visually as the smallest amount of drug that still produced a clearly distinguishable inhibition zone around the edges of tilmicosin contained pores on nutrient agar media was 0.20 µg/ml.

Pharmacokinetics and statistical analysis

Serum concentrations of tilmicosin versus time data obtained during the study were utilized for calculating various pharmacokinetic variables using a compartmental and non-compartmental analysis using computerized program, WinNonline 4.1 (Pharsight, USA).

The peak concentrations (C_{max}) and time to peak (T_{max}) were obtained from the serum concentration-time data directly. The areas under the serum concentration of tilmicosin time curves from time 0 to the last sample collected (AUC_{0-24}) were calculated using linear trapezoidal method.[12] While $AUC_{0-\infty}$ was derived from AUC_{0-24} and $AUC_{24-\infty}$, where $AUC_{24-\infty}$ was equal to C_{24}/ß. For bio-equivalence evaluation, the ratios of C_{max} (T/R), AUC_{0-24} (T/R) and $AUC_{0-\infty}$ (T/R) were calculated. Values within the bio-equivalence acceptable range at 90% confidence interval, 0.80 to 1.25 were considered for accepting the null hypothesis of bio-equivalence between the reference and the test brands.[13,14]

RESULTS

The mean serum concentrations of tilmicosin in Micotil 300® and Cozina 300® following oral administration of 15 mg tilmicosin/kg body wt. in broiler chickens are shown (Table 1).

Table 1: Serum concentrations (µg/ml) of tilmicosin in Micotil 300® and Cozina 300® following oral administration of 15 mg tilmicosin/kg body wt. in broiler chickens (n=12).

Time post administration (hour)	Mean serum concentration (µg/ml)	
	Micotil 300® (reference)	Cozina 300® (test)
0.08	0.19±0.003	0.16±0.002
0.16	0.28±0.002	0.25±0.002
0.25	0.43±0.01	0.39±0.01
0.5	0.92±0.06	0.87±0.05
1	1.52±0.09	1.46±0.08
2	1.99±0.08	1.91±0.07
4	1.26±0.06	1.23±0.03
6	0.93±0.06	0.87±0.03
8	0.75±0.03	0.69±0.02
12	0.53±0.03	0.48±0.01
24	0.24±0.001	0.21±0.002

Mean (X±S.E).

Table 2: Pharmacokinetic parameters of tilmicosin in Micotil 300® and Cozina 300® following oral administration of 15 mg tilmicosin/kg body wt. in broiler chickens (n=12).

Parameter	Unit	Micotil 300® (reference)	Cozina 300® (test)
K_{ab}	h^{-1}	1.10±0.07	1.03±0.03
K_{el}	h^{-1}	0.070±0.001	0.073±0.001
$t_{1/2(ab)}$	h	0.62±0.05	0.66±0.03
$t_{1/2(el)}$	h	9.89±0.12	9.48±0.17
C_{max}	µg/ml	1.73±0.08	1.67±0.05
t_{max}	h	2.01±0.13	2.04±0.03
AUC	µg/ml/h	20.31±1.29	18.62±1.08
AUMC	µg/ml/h	263.21±17.03	228.43±14.94
MRT	h	12.95±0.42	12.26±0.39

Mean (X±S.E). k_{ab}; K_{el} absorbtion and elimination rate constant after oral administration; $T_{0.5(ab)}$ absorbtion half-life after oral administration; $T_{0.5(el)}$ elimination half-life after oral administration; C_{max} maximum plasma concentration; T_{max} time to peak plasma concentration; AUC; area under serum concentration-time curve; AUMC area under moment curve; MRT mean residence time.

The mean pharmacokinetic parameters of tilmicosin in Micotil 300® and Cozina 300® after oral administration of 15 mg tilmicosin/kg body wt. in broiler chickens are shown (Table 2).

The disposition kinetics of tilmicosin in Micotil 300® and Cozina 300® following oral administration of 15 mg tilmicosin base/kg body wt. revealed that the maximum blood concentration [C_{max}] were 1.73 and 1.67 µg/ml and attained at [T_{max}] of 2.01 and 2.04 hours, respectively. The mean ratio of C_{max} and AUC of the tested and reference formulations were within bio-equivalence range and summarized in Table 3.

Table 3: Bio-equivalence between Micotil 300® (reference) and Cozina 300® (test) formulations.

Bio-equivalence	C_{max}	AUC_{0-24}	$AUC_{0-\infty}$
Micotil 300® (reference)	1.73±0.08	16.89±1.09	20.31±1.29
Cozina 300® (test)	1.67±0.05	15.75±0.94	18.62±1.08
Point estimate	0.96	0.93	0.91
Acceptable range	0.80-1.25	0.80-1.25	0.80-1.25
Conclusion	BE	BE	BE

BE-bio-equivalence.

The bio-equivalence ratio for mean C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ (T/R) of Cozina 300® versus the reference product (Micotil 300®) were 0.96, 0.93 and 0.91 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80 to 1.25 (U.S. Food and Drug Administration, 2003). The two oral formulations of tilmicosin (Micotil 300® and Cozina 300®) in this experiment could therefore be considered bioequivalent.

All the experimental chickens remained healthy during and after the study.

DISCUSSION

The clinical efficacy of an antimicrobial is determined not only by its activity against infective organisms but also by its ability to reach the site of infections and its persistence within tissues. Pharmacokinetic variables such as plasma concentration, half life, bioavailability, rate of elimination are important considerations for rational use of antimicrobial agents.

After intravenous administration of tilmicosin in broiler chicken, cardiovascular toxicity and deaths had been mentioned.[15,16] For this reason, we can't calculate the bioavailability of tilmicosin in my study.

In the present study, oral administration was used due to the fact that this is the common procedure employed in poultry farms. The value of C_{max} determined in this study after an oral dose of 15 mg tilmicosin/kg b.wt. (1.73 and 1.67 µg/ml for Micotil 300® and Cozina 300®, respectively. This finding was lower than that recorded for tilmicosin in chicken 2.09 µg/ml, while this quality was higher than reported in swine 1.19 µg/ml.[17,18]

On the other hand, time to peak serum concentration was (2.01 and 2.04 h, for Micotil 300® and Cozina 300®, respectively). This result was nearly similar to that recorded for tylosin in chicken 2.36 h.[19]

While it was shorter than the result recorded for tilmicosin in chicken 3.99 h.[17] In this study, the calculated AUC_{0-24} was observed to be 16.89 and 15.75 µg.h/ml, for Micotil 300® and Cozina 300®, respectively. The obtained result is lower than that recorded for tilmicosin in chicken (21.82 $\mu g.h.ml^{-1}$,[17] and similar to tylosin in broiler chicken (18.60 $\mu g.h.ml^{-1}$.[20] In any case, the area under serum concentration-time curve reported in this study is higher than rates reported in pig (9.68 $\mu g.h.ml^{-1}$.[21] Such contrasts are regular and habitually identified with interspecies variety, examine strategies utilized, age, breed and wellbeing status of the creature, and the plan of the medication utilized.

The elimination half-lives of tilmicosin ($t_{0.5el}$) were 9.89 and 9.48 h, for Micotil 300® and Cozina 300®, respectively. These results are higher than that recorded for tilmicosin in broiler chickens (7.30 h).[22]

The effectiveness of a drug is partly dependent on its formulation, route of administration and metabolic pattern. These factors determine the plasma concentration-time profile of the drug. Following administration of a single oral dose (15 mg/kg b.wt) of tilmicosin formulations to healthy broiler chickens, therapeutic concentration were achieved 5 minutes post administration in all the chickens. Concentration of tilmicosin in serum from 5 min up to 24 h exceeds the MIC against sensitive micro-organisms. The concentration was detected up to 24 hours in the serum of chickens given (Micotil 300® as a reference product and Cozina 300® as a tested product) above the MIC for *M. gallisepticum* (0.027-0.15 µg/ml).[23]

The area under the curve (AUC) estimation, using the method of trapezoids, is the critical step in the calculation of pharmacokinetic estimations using non-compartmental analysis.[24]

Bio-equivalence study is a test to assure the clinical efficacy of a generic versus brand drugs.[9] Bio-equivalence refers to a comparison between generic formulations of a drug, or a product in which a change has been made in one or more of the ingredients or in the manufacturing process, and a reference dosage form of the same drug. This study shows that the bio-equivalence ratio for mean C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ (T/R) of Cozina 300® versus the reference product (Micotil 300®) were 0.96, 0.93 and 0.91 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80 to 1.25.[25]

The two oral formulations of tilmicosin (Micotil 300® and Cozina 300®) in this experiment could therefore be considered bioequivalent.

CONCLUSION

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that Cozina 300® which manufactured by Boston Company, Pharma Cure Division, Egypt, is bioequivalent to Micotil 300® which manufactured by Elanco-Animal Health Gmbh, Germany) and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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